REMARKS

I. Overview

Applicant's have reviewed and considered the office action dated June 29, 2005 and the cited references therewith. Claim 1-73 are pending in this application.

Applicants note that claims 48, 49 and 51-73 claiming a polynucleotide construct were found to be allowable. Thus, Applicants respectfully submit that newly added claims 76-80 directed towards methods of using the allowed compositions of claims 48, 55, 62, 68 and 72 are likewise allowable.

Claims 12-14, 16-17, 26-28, and 74-75 have been cancelled. Claims 1, 9, 10, 23-24, 35-36, 38, 40, 50 and 57 have been amended. Support for these amendments may be found in the Published Specification at paragraphs 8, 13, 39, 72, 74, 105, 106, 114, and Figures 1A and 1B. No new matter has been added. The present response is an earnest effort to place all claims in proper form for immediate allowance. Reconsideration and passage to issuance is therefore respectfully requested.

II. Specification

A. The Examiner writes that the disclosure is objected to because of the following informalities: The Saha and Weeraratna references of page 2 are incorrectly referenced.

The Applicant's thank Examiner for pointing out this inadvertent error and accordingly the specification has been amended at page 2, lines 23 so that it now recites the following:

"Using the transcriptome to annotate the genome", Saha et al., Nature Biotech 2002 20: 508-512; "Generation and analysis of melanoma SAGE libraries: SAGE advice on the melanoma transcriptome", Weeraratna et al, Oncogene 2004 23(12):2264-2274."

B. The Examiner writes that the disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. The Examiner writes that the Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code pursuant to MPEP § 608.01.

Applicant's thank Examiner for pointing out this inadvertent error and accordingly have amended the specification at page 18, line 14 and at page 31, line 13 so that the sentences now recite "Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology-Information (http:// world wide web at ncbi.nlm.nih.gov/)."

C. The Examiner writes that the application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid set forth in 37 CFR 1.821(a)(1) and (a)(2). Examiner writes according to 37 C.F.R. 1.821 any peptide sequence of greater than four amino acids must appear in the sequence listing. The Examiner notes at page 38 sequences of both the c-myc and FLAG epitope tags are disclosed.

Applicants thank Examiner for pointing out this inadvertent error and have accordingly amended the specification at page 38, line 4 so that it now recites "c-myc epitope tag (peptide seq. EQKLISEEDL) (Stratagene) (SEQ ID NO: 10), FLAG epitope tag (peptide seq. DYKDDDDK) (Stratagene) (SEQ ID NO: 11)". In addition, Applicants have submitted a sequence listing in compliance with 37 C.F.R. 1.821.

In light of the above, Applicants respectfully request the objections to the Specification be withdrawn.

III. Claim Objections

A. Claims 12-14, 16-17, and 26-28 stand objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim.

Claims 12-14, 16-17, and 26-28 have been canceled rendering this rejection moot.

B. The Examiner writes that Applicant is advised that should claim 27 be found allowable, claim 28 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof.

Claims 27-28 have been canceled rendering this rejection moot.

IV. Claim Rejections Under 35 USC Section 112

Claims 74 and 75 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner writes that the claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 74 and 75 have been cancelled rendering the rejection on this basis moot.

V. Claim Rejections 35 USC Section 112

A. Claims 1-47 stand rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The Examiner writes that the omitted steps are: in independent claim 1, 10, 24, 36, 38, and 40 an mRNA isolation step is required to link the first portion of the claim describing the structure of the introduced constructs t the method steps that follow, starting with the step of "reverse transcribing the isolated mRNA." Examiner writes that claim 1 additionally requires a step that is missing prior to the step of "self-ligating the cDNA fragment."

Claims 1, 10, 24, 36, 38, and 40 have been amended so that they now require "isolating mRNA from said cell". Support for this amendment can be found in the Published Specification at paragraph 8. In addition, claim 1 now requires "subjecting said cDNA fragment with a DNA

polymerase and nucleotides to generate a blunt-ended fragment". Support for this amendment can be found in the Published Specification at paragraph 114.

Applicants disagree with the Examiner's statement that claims 10, 24, 38, and 40 further lack essential elements pertaining to the linkers to be ligated to the Type IIS cleaved fragments. The present invention of elucidating a RNA transcription profile in a eukaryotic cell does not require a linker containing a restriction enzyme restriction (RER) site for later cleavage steps to practice the claimed method. Rather, a linker may contain a RER site or the RER site may be incorporated in the primer used to amplify the products by PCR. Alternatively, a linker or a primer used for the PCR amplification may not contain a restriction site at all, as the amplified products can be cloned in a blunt-end fashion on the linker side. Thus, having no restriction site on the linker side does not prevent individual cloning of fragments, or the subsequent concatamerization of amplified units prior to cloning (concatamers will have a head-head/tail-tail/head-head/tail-tail structure).

The second issue raised by the Examiner is that the linker has to have an appropriate number of randomized overhang nucleotides to allow for ligation of the linkers to the unidentified overhangs produced by Type IIS enzyme digestion. Applicants disagree. A person skilled in the art would be familiar with cloning strategies and be aware that while sticky-ended ligation using compatible cohesive ends generated by restriction enzymes is one possibility, it would not preclude the possibility of cloning cDNA fragments that were subjected to Type IIS digestion followed by the ligation of linkers or blunt-end cDNA fragments generated for example by PCR and their subsequent ligation into a sequencing vector.

Therefore, claims 1-47 have not omitted essential steps and therefore are complete. Applicants respectfully request that the rejection to claims 1-47 be withdrawn and reconsidered.

B. Claims 1-47 and 50 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner writes that there is insufficient antecedent basis for the limitation "the isolated mRNA" in the claims 1, 10, 24, 36, 38, and 40. The Examiner writes that if the additional step of isolating the mRNA were added to the claims as required above, this would correct this antecedent basis problem.

Claims 1, 10, 24, 36, 38, and 40 have been amended so that they now require "isolating mRNA from said cell". Support for this amendment can be found in the Published Specification at paragraph 8. Applicants respectfully submit that there is now sufficient antecedent basis and independent claims 1, 10, 24, 36, 38, 40, and 48 are definite and claims 2-9, 11, 15, 18-23, 25, 29-35, 39, 41-47, and 50 dependent therefrom are likewise definite. Applicants respectfully request that the rejection to claims 1, 10, 24, 36, 38, and 40 be withdrawn and reconsidered.

C. Claims 1, 10, 12-13, 24, 26-28, 36, 38, and 40 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for reciting two restriction sites at both the 5' end and the 3' end, that "at least one of the RER sites is recognized by a Type IIS restriction enzyme." Examiner writes that this is vague and indefinite because it implies that the other RER site could also be Type IIS enzyme. The examiner also states for either insertion of a fragment into a sequencing vector or concatamerization of the fragments prior to insertion into a sequencing vector to successfully take place the overhangs produced must be known and have an appropriate cohesiveness. As a Type IIS enzyme leaves overhangs that would differ for almost any two different Type IIS recognition sites they would not be an effective choice for the second RER site present at each end of the marker.

Applicants respectfully disagree as one or both RER sites located at the exon boundary could indeed be a Type IIS restriction enzyme recognition site. Applicants have amended claim 1 so that it recites "two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme", "two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme", and "subjecting the cDNA to digestion with one or more Type IIS restriction enzymes that recognize one Type IIS RER site located at the 5' end of the marker exon and one Type IIS RER site located at the 3' end of the marker exon". Independent claims 10, 24, 36, 38, and 40 have been similarly amended. Support for this amendment can be found in the Published Specification at Figures 1A and 1B.

The first digestion (the one that cuts within the unknown sequence and outside the maker exon) has to be performed with a Type IIS restriction enzyme. The second digestion (the one that cuts within the marker exon) can be performed with a Type IIS restriction enzyme (different from the one used first) or a regular Type II enzyme depending on the researcher preference and the particular sequence of the exon marker boundaries.

When a Type IIS enzyme cuts within a known sequence (such as within the marker exon) the cohesiveness of the ends will be known for all molecules in the mix, and appropriate ligation or concatamerization methods, commonly know in the art (such as direct ligation, use of adapters or blunt-ending), can be designed according to the particular sequence of the marker exon sequence boundaries. Therefore, the methods of the present invention can be implemented by using a Type IIS restriction enzyme as the second enzyme. Therefore, the phrase "one or more

Type IIS restriction enzymes that recognize one Type IIS RER site located at the 5' end of the marker exon and one Type IIS RER site located at the 3' end of the marker exon" is not vague or indefinite. In light of the remarks above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection to claims 1, 10, 24, 36, 38, and 40.

E. Claim 10 recites the limitation "subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER sites located at the 5' end of the marker exon." The Examiner writes that this is vague and indefinite because it is unclear what is fully intended by this limitation as only one Type IIS restriction site has been identified previously at the 5' end. The Examiner writes to delete "one of" would be remedial. Claim 10 further recites the limitation "subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzymes." The Examiner writes that this limitation should recite something along the lines of –subjecting the amplification products to one or more restriction enzymes that recognize the RER site not previously recognized by the Type IIS restriction enzymes and that recognize the RER site present in the ligated linker sequence--. The Examiner writes that claims 24, 38, and 40 have these same issues.

As stated in the above previous rejection to claims 1, 10, 12-13, 24, 26-28, 36, 38, and 40, claim 10 is thought to be definite and clear. Claim 10 has been amended so that it recites "subjecting the cDNA to a Type IIS restriction enzyme that recognizes one Type IIS RER site located at the 5' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon". Support for this amendment can be found in the Published Specification at Figures 1A and 1B.

One or both RER sites located at the exon boundary could be a Type IIS restriction enzyme recognition site. The first digestion (the one that cuts within the unknown sequence and outside the maker exon) has to be performed with a Type IIS restriction enzyme. The second digestion (the one that cuts within the marker exon) can be performed with a Type IIS restriction enzyme (different from the one used first) or a regular Type II enzyme depending on the researcher preference and the particular sequence of the exon marker boundaries.

When a Type IIS restriction enzyme cuts within a known sequence (such as within the marker exon) the cohesiveness of the ends will be known for all molecules in the mix, and appropriate ligation or concatamerization methods, commonly know in the art (such as direct ligation, use of adapters or blunt-ending), can be designed according to the particular sequence of the marker exon sequence boundaries. Therefore, the methods of the present invention can be implemented by using a Type IIS restriction enzyme as the second enzyme. Therefore, the phrase "subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER sites located at the 5' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon" is not vague or indefinite. In light of the remarks above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection to claim 10.

F. Claims 36, 38, and 40 recite the limitation "the polynucleotide construct" in line 3. The Examiner writes that there is insufficient antecedent basis for this limitation in the claims.

Accordingly, the phrase "the polynucleotide construct" has been removed from claims 36, 38, and 40 and replaced with the phrase "the polynucleotide marker fragment". Support for this amendment can be found in the Published Specification at paragraph 105. In view of the foregoing amendment, Applicants respectfully request that the Examiner withdraw the rejection to claims 36, 38, and 40.

G. The Examiner writes that claim 36 is vague and indefinite because it recites "RNA tags." The Examiner writes that it is unclear what is intended by this limitation as the tags in question are not composed of RNA, but instead consist of cDNA. The Examiner writes thus the skilled artisan would be unable to determine the metes and bounds of this claim limitation.

Claim 36 has been amended so that the phrase "RNA tags" has been replaced with "nucleotide sequence tags". Support for this amendment can be found in the Published Specification at paragraphs 13 and 74. In view of the foregoing amendment, Applicants respectfully request that the Examiner withdraw the rejection to claim 36.

H. The Examiner writes that claims 9, 23, and 35 recite that the fluorescent protein will be "measured by fluorescence activated flow cytometry." This is unclear because "fluorescence activated flow cytometry" is not an art-recognized term. The art-recognized terms are either - -fluorescence-activated cell sorting--, or –flow cytometry--. It would seem that in the instant case, the appropriate term would be to recite –flow cytometry--.

Applicants have adopted the Examiner's suggestion so that the phrase "fluorescence activated flow cytometry" has been replaced with "flow cytometry". Support for this amendment can be found in the Published Specification at paragraph 106. In view of the foregoing amendment, Applicants respectfully request that the Examiner withdraw the rejection to claim 9, 23, and 35.

I. Claim 50 recites that the polynucleotide construct further comprises a "positive selection marker." The Examiner writes that this is unclear because the specification only teaches of the use of a positive selection marker, such as GFP, as the marker exon and a negative selection marker, such as neomycin resistance, downstream of the marker exon cassette. The Examiner writes thus, in light of the specification, the skilled artisan would be unclear about the

metes and bounds of this limitation since the disclosure only seems to teach the placement of a negative selection marker downstream of the marker exon not a positive selection marker. The Examiner suggests to change "positive selection marker" to - -negative selection marker- - would be remedial.

Applicants respectfully disagree with the Examiner's suggestion that the neomycin resistance gene is a "negative selection marker". A negative selection marker is used to kill cells that express the marker. In contrast, a positive selection marker is used to select for survival or for purification of cells that express the marker. However, in order to expedite prosecution, claim 50 has been amended by replacing the phrase "positive selection marker" with the phrase "selection marker". Support for this amendment can be found in the Published Specification at paragraph 72. In view of the foregoing amendment, Applicants respectfully request that the Examiner withdraw the rejection to claim 50.

VI. Conclusion

Enclosed is our check for $500.00 for 5 new independent claims. No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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